

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
27 May 2004 (27.05.2004)

PCT

(10) International Publication Number
WO 2004/044584 A1

(51) International Patent Classification?: **G01N 33/542**,
33/566, 33/569, C07K 16/28, G01N 15/14

(21) International Application Number:
PCT/EP2003/012664

(22) International Filing Date:
12 November 2003 (12.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02025335.7 13 November 2002 (13.11.2002) EP

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR,
CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE,
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: METHOD FOR IDENTIFYING ANTIGEN SPECIFIC B CELLS



(57) Abstract: The present invention relates to a method of identifying a B cell carrying a surface immunoglobulin molecule having a binding site for an antigen of interest comprising contacting a sample putatively containing said B cell with the antigen of interest wherein said antigen is labeled with a first label and with a receptor specifically binding to said surface immunoglobulin molecule wherein said receptor is labeled with a second label and wherein said first label, when being brought into a spatial proximity of between 10 and 100 Angstrom with said second label emits a detectable signal upon activation of said second label by an external source and assessing the presence of said detectable signal, wherein said presence is, in turn, indicative of the B cell carrying a surface molecule having a binding site for the antigen of interest.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/12664

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/542 G01N33/566 G01N33/569 C07K16/28 G01N15/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>TOWNSEND S E ET AL: "Single epitope multiple staining to detect ultralow frequency B cells"</p> <p>JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 249, no. 1-2, 1 March 2001 (2001-03-01), pages 137-146, XP004317478</p> <p>ISSN: 0022-1759</p> <p>page 139; figure 2</p> <p style="text-align: center;">--- -/--</p>	1-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

16 April 2004

Date of mailing of the international search report

26/04/2004

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KUBITSCHKE U ET AL: "FLUORESCENCE RESONANCE ENERGY TRANSFER ON SINGLE LIVING CELLS APPLICATION TO BINDING OF MONOVALENT HAPTENS TO CELL-BOUND IMMUNOGLOBULIN E" BIOPHYSICAL JOURNAL, NEW YORK, US, US, vol. 60, August 1991 (1991-08), pages 307-318, XP000882076 ISSN: 0006-3495 page 311	1-25
A	KOKSCH M ET AL: "Fluorescence resonance energy transfer as a new method for the epitope-specific characterization of anti-platelet antibodies" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 187, no. 1, 16 November 1995 (1995-11-16), pages 53-67, XP004020967 ISSN: 0022-1759 page 57-60	1-25
A	LUNDIN K ET AL: "Development of a time-resolved fluorescence resonance energy transfer assay (Cell TR-FRET) for protein detection on intact cells" ANALYTICAL BIOCHEMISTRY, ORLANDO, FL, US, vol. 299, no. 1, 1 December 2001 (2001-12-01), pages 92-97, XP002269887 ISSN: 0003-2697 figure 1	1-25
A	US 5 213 960 A (CHANG TSE W) 25 May 1993 (1993-05-25) column 7, line 45 -column 8, line 25	1-25
X	US 2002/006403 A1 (YU XUE-ZHONG ET AL) 17 January 2002 (2002-01-17) page 1, paragraph 12	26
X	WO 00/26667 A (MILLER JONATHAN L) 11 May 2000 (2000-05-11) figure 2; examples SEQ,44	27
X	DE 100 19 967 A (FENNING BIOMED GMBH DR) 25 October 2001 (2001-10-25) examples SEQ,7-8	27
X	WO 01/98361 A (GENENTECH INC ;DEVAUX BRIGITTE (US); HONGO JO ANNE S (US); SHELTON) 27 December 2001 (2001-12-27) examples SEQ,8	27
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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/29538 A (BECTON DICKINSON CO) 26 April 2001 (2001-04-26) page 8-10; figure 2 -----	28

INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28

method and tools for identifying antigen-specific B cells

1.1. Claims: 1-25

method for identifying a B cell carrying a surface immunoglobulin

1.2. Claim : 26

antibody specific for human CD28

1.3. Claim : 27

antibody comprising an amino sequence selected from the group consisting of SEQ ID No 76,78,80,82,84,86 and 88 and/or which is encoded by a nucleic acid sequence comprising a sequence selected from the group SEQ ID No 77,79,81,83,85,87,89,60 and 61.

It is noted that this subinvention can be also splitted in different sub-subinventions because some of the above sequences are already known from the state of the art.

1.4. Claim : 28

flow cytometer device

It is not apparent at the present stage what can be taken as common novel and inventive concept within the sense of Rule 13 PCT for linking the subject-matter of independent claims 1, 26, 27 and 28.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/12664

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